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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application: Clayton et al.

Serial No.: 10/049,142 Examiner: M. Bahar

Filing Date: 5 Feb 02 Art Unit: 1617

For: USE OF EP4 RECEPTOR LIGANDS IN THE TREATMENT OF NEUROPATHIC PAIN AND COLON CANCER

Director of the United States Patents and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

Rule 132 Declaration of Nicholas Maughan Clayton, B.Sc.

Sir:

I, Nicholas Maughan Clayton do hereby declare and say as follows:

1. I am a Principle Scientist in the area of pain research, at GlaxoSmithKline Inc., Harlow, New Frontiers Science Park N&S Third Avenue, Essex CM 19 5AW, United Kingdom. Attached hereto as Exhibit A is my curriculum vitae.

2. I am an inventor of U.S. Patent Application Serial No. 10/049,142, filed 5 February 2002 (hereinafter the "instant Application") and have read the application.

3. I have read the comments from the U.S. Patent Examiner set forth in the Office Action mailed 2 October 2003, and particularly the comments under the heading "Claim Rejections -35 U.S.C. §103". For the reasons described below, I respectfully disagree with the Examiner's conclusion that the invention in my patent application is obvious.

4. I performed and directed experiments designed to evaluate the effect of a COX-2 inhibitor and an EP4 ligand, each alone and in combination,

on reducing the hypersensitivity to pain induced by Freunds Complete Adjuvant (FCA). This experiment is a recognized and appropriate model for evaluating efficacy of a compound for the treatment of inflammatory pain. The experiments employed 2-(4-Ethoxyphenyl)-3-(4-methanesulfonyl-phenyl)-pyrazolo[1,5-b]pyridazine (hereinafter "COX-2 inhibitor") as the COX-2 inhibitor and [4-(4,9-Diethoxy-1-oxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl)phenyl]acetic acid (hereinafter "EP4 ligand) as the EP4 ligand. The COX-2 inhibitor is described in U.S. Patent No. 6451794, issued 17 Sep 2002, and published on 18 Mar 1999 as PCT Publication No. WO99/12930. The EP4 ligand is described in the instant Application at page 8, lines 14-20, respectively. In this single dose study, the analgesic effect of each compound alone was compared to the combination of both. In my view, the comparison is the closest comparison that could be made to address the comments of the Patent Examiner.

5. I employed the following experimental methods to measure analgesic effect based on weight bearing sensitivity to pain.

Weight Bearing Study Methods

Inflammatory hypersensitivity to pain was induced in groups of seven (7) rats by intraplantar injection of FCA (100 µl, 1 mg/ml) into the left hind paw. 23 Hours after administration of FCA the animals were dosed as described below for each experiment and group.

Experiment 1

Group 1 --Control

Vehicle for EP4 ligand p.o. then 1 hour later vehicle for COX-2 inhibitor p.o.

Group 2 –EP4 Ligand Alone

EP4 ligand 0.1 mg/kg p.o. then 1 hour later vehicle for COX-2 inhibitor p.o.

Group 3 –COX-2 Inhibitor Alone

Vehicle for EP4 ligand then 1 hour later COX-2 inhibitor 0.3 mg/kg p.o.

Group 4 –Combination EP4 Ligand and COX-2 Inhibitor

EP4 ligand 0.1 mg/kg p.o. then 1 hour later COX-2 inhibitor 0.3 mg/kg p.o.

Experiment 2

Group 1 --Control

Vehicle for EP4 ligand p.o. then 1 hour later vehicle for COX-2 inhibitor p.o.

Group 2 –EP4 Ligand Alone

EP4 ligand 0.03 mg/kg p.o. then 1 hour later vehicle for COX-2 inhibitor p.o.

Group 3 –COX-2 Inhibitor Alone

Vehicle for EP4 ligand then 1 hour later COX-2 inhibitor 0.1 mg/kg p.o.

Group 4 –Combination EP4 Ligand and COX-2 Inhibitor

EP4 ligand 0.03 mg/kg p.o. then 1 hour later COX-2 inhibitor 0.1 mg/kg p.o.

One hour after the second dose the effect on the established FCA-induced decrease in weight bearing (hypersensitivity) was determined. Normal rats distribute their body weight equally between the two hind paws. When the left hind paw is inflamed and/or painful, the weight is re-distributed so that less weight is put on the affected paw, hence a decrease in weight bearing. Assessment of this change is a sensitive method of measuring allodynia and incident pain and is described in the literature. See, Clayton et al., *British Journal of Pharmacology* **120**: P78 (1997).

6. From the above-described experiments, I obtained the following results expressed as a percentage reversal of the decrease in weight bearing with respect to controls.

FCA hypersensitivity to pain was established.

Intraplantar FCA produced a significant decrease in weight bearing on the inflamed left hind paw 23 hours post administration.

Experiment 1

The EP4 ligand (0.1 mg/kg p.o.) and the COX-2 inhibitor (0.3 mg/kg p.o.) each alone had no significant effect on the hypersensitivity to pain. The combination of EP4 ligand and COX-2 inhibitor produced a synergistic effect with a 79% inhibition of hypersensitivity. The results for Experiment 1 are reported graphically on Exhibit B Figure 1.

Experiment 2

The EP4 ligand (0.03 mg/kg p.o.) and the COX-2 inhibitor (0.1 mg/kg p.o.) each alone had no significant effect on the hypersensitivity to pain. The combination of EP4 ligand and COX-2 inhibitor produced a synergistic effect with an 82% inhibition of hypersensitivity. The results for Experiment 2 are reported graphically on Exhibit B, Figure 2.

7. I conclude that the two experiments demonstrate a synergistic effect on hypersensitivity to pain when the EP4 ligand and the COX-2 inhibitor are used in combination because the mean plus sem of the two compounds alone added together is less than the mean plus the sem of the combination of the two.

8. The synergistic effect of this combination of an EP4 ligand and a COX-2 inhibitor was unexpected because I could not have predicted, based upon the two distinct mechanisms of action of these compounds, that the results would show synergy.

9. I hereby declare that all statement made of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that theses statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Nicholas Maughan Clayton

13/2/2004
Date

Date: 10 February, 2004
GlaxoSmithKline Inc.
Five Moore Drive, PO Box 13398
Research Triangle Park
North Carolina 27709

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Director of the United States Patent and Trademark Office, P.O. Box 1450, Alexandria, VA, 22313-1450 on Feb. 13, 2004 in accordance with the provisions of 37 CFR § 1.8.

Valerie L. Phillips
Valerie Phillips

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Exhibit A

NICK CLAYTON

GlaxoSmithKline Inc., Harlow, New Frontiers Science Park N&S
Third Avenue, Essex CM 19 5AW, United Kingdom

A senior Pharmacologist with extensive experience working for a major, blue chip pharmaceutical company. A high level of scientific and technical experience in the area of gastrointestinal pharmacology and pain.

Started at Glaxo Jan 2nd 1978 . Title Junior Scientist
B.Sc. June 1983
Promoted to Phamacologist October 1983
Promoted to Senior Pharmacologist April 1991
Promoted to Principle Scientist 2000

From 1978-1995 my work has focused in the area of Gastrointestinal Pharmacology. Since 1995, my work has focused in the area of Pain research.

KEY ACHIEVEMENTS/SKILLS

- Directed, supervised and carried out the GLP studies on inhibition of acid secretion, serum gastrin levels and gastric hyperplasia in conscious gastric fistula rats in collaboration with Toxicology. These studies demanded a high degree of accuracy and were carried out in strict accordance to GLP rules and regulations. In addition these series of studies demanded very good networking skills as several departments and divisions were involved.

- Helped set up the inflammatory diseases project, the aim of which was to develop animal models to investigate the mechanism by which NSAIDs induced gastric and intestinal ulcers.

- Set up two *in vitro* screens; isolated guinea-pig right atria and trachea to look at the selectivity of β_3 agonists.

- Used radio-immune and enzyme immuno-assays to measure prostaglandin's and leukotriene levels from blood and tissue.

- Developed a rat anti-neutrophil antibody.

- Developed a model of sensitised small intestine measured as a increase in transit.

- Initiated and directed two Clinical Collaborations to investigate the aetiology and pathophysiology of Non-Ulcer Dyspepsia and Irritable Bowel Syndrome, in association with Clinical Pharmacology, Chemotherapy, Histopathology, Northwick Park Hospital, London and Meath Hospital, Dublin.

  These clinical collaborations have demanded excellent inter-personal and networking skills to ensure the smooth running of these studies and a detailed understanding of the clinical aspects of the disease.

- GlaxoWelcome supervisor for BBSRC PhD studentships at Sheffield University and a post Doc

- Developed a readout of visceral pain in the conscious rat based on the electromyograph activity (EMG) of the abdominal oblique muscles and have also developed a method of sensitising the colon which is observed as a reduced threshold for visceral pain.
- Developed a novel method of measuring small intestinal and colonic motility using telemetry.
- Moved into the area of pain 8 years ago. Now working on models of inflammatory, visceral and neuropathic pain.
- Last 2 years Group leader of the inflammatory pain in-vivo group. Line manager of two people
- Oversee target validation of novel targets for the treatment of inflammatory pain
- Responsible for ensuring the testing and progression of novel analgesics from the primary screen stage to candidate selection. Provide pre-clinical support for development compounds. In this capacity on three programme teams
- Spent a week (July 1999) in a specialist pain clinic (Norwich Hospital) to increase my awareness about the problems, issues around treating patients with chronic pain

TECHNICAL/SCIENTIFIC EXPERTISE

- 26 years experience in *in- vivo* pharmacology and in the last eight years have also become heavily involved in setting up and running models of inflammatory pain
- Technically expert in the use of behavioural models of pain
- Analgesia project licence holder. Administration of personnel licences for people in the project up to expiry of licence (2001) Now deputy project licence holder for new analgesia project licence
- Surgical procedures including implanting electrodes, gastrointestinal cannula, gastric fistula etc in both recovery and non-recovery animals.
- Wide experience in the use of a large number of *in vivo* animal models, including models of gastrointestinal inflammation and ulceration, gastric acid secretion, gastrointestinal motility and transit, visceral pain, cutaneous pain and in anaesthetised rats measurement of blood pressure.
- *In vitro* preparations including Guinea pig atria.
- Various biochemical procedures including radioimmunoassays, enzyme immunoassays, blood cell counting and the Cutaneous passive anaphylaxis test.
- In-depth knowledge of gastrointestinal inflammation, ulceration, Irritable Bowel Syndrome, gastrointestinal motility, visceral pain and cutaneous pain.

SUPERVISORY, MANAGEMENT AND LEADERSHIP EXPERIENCE

- Extensive supervisory experience of the last 21 years.
- Group leader for the inflammatory pain in-vivo group.
- In the last year have become involved in developing a strategy for IBS, embracing both clinical studies and basic research.
- Recently been heavily involved in redesigning the company strategy for developing novel analgesics

- Closely involved in developing a research and clinical plans for a number of novel analgesics

Fisons Agrochemical Division **1974 – 1978**

EDUCATION AND QUALIFICATIONS

Hatfield Polytechnic BSc *Thesis*	Applied Biology - Upper 2nd class honours *'A study into the interaction of propranolol With histamine in the isolated guinea-pig lung strip.'*	1979 - 1983
HNC	Applied Biology	1977 - 1979
ONC	Applied Biology	1975 - 1977

PUBLICATIONS

Full Papers

O'Sullivan M O, Clayton N, Breslin NP, Harman I, Bountra C, Mclaren A, Morain Ca. (1999) Increased mucosal mast cells in the irritable bowel syndrome. Submitted to Neurogastroentrology and Motility

Baxendale, A, Bountra, Clayton, N, Gunput, Humphrey, PPA, Kozlowski, K, Mangel A, & O'Sullivan M. (1999). Irritable bowel syndrome as visceral hyperalgesia: implications for therapy. Current Opinion in Central & and Peripheral Nervous System Investigational Drugs, 1 (1) 86-97.

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Clayton NM, Oakley I, Williams LV and Trevethick MA. (1996). The role of acid in the pathogenesis of Indomethacin induced gastric antral ulceration in the rat. Alimentary Pharmacology and Therapeutics, 10, 339-345.

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Trevethick MA, Clayton NM, Strong P, Harman IW. (1993). Do infiltrating neutrophils contribute to the pathogenesis of indomethacin induced ulceration of the rat gastric antrum? Gut, 34, 156-160.

Anthony A, Clayton NM, Dhillon AP, Nygard G, Hudson M, Piasecki C, Strong P, Trevethick MA, Jordan CC, Pounder RE, and Wakefield AJ. (1993). Early histological features of small intestinal injury induced by indomethacin. Aliment Pharmacol Ther, 7, 29-40.

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Bunce KT and Clayton NM. (1987). The effects of the stable thromboxane A2-mimetic U46619, on gastric mucosal damage and gastric non-parietal secretion in the rat. Br J Pharmacol, 91, 23-29.

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Abstracts

S.D. Collins, N Clayton, M. nobbs, C. Bountra. The effect of Lamotrigine, Gabapentin and Morphine on neuropthic pain in the rat. Poster at 9th World Congress on pain in Vienna. August 1999

N Cougnon-Aptel, N Clayton, T Brown, R Munglani, C Bountra, K Dale. Biphasic induction of COX-2 in the spinal cord in a model of chronic inflammatory hyperalgesia Poster at 9th World Congress on pain in Vienna. August 1999

N Clayton, J Francis, R Munglani, S Collins, C Bountra The Novel Sodium Channel Blocker 4030W92 reduces the fall in CGRP expression in the dorsal horn of the spinal cord and prevents the development of mechanical allodynia following chronic constriction injury (CCI) in the rat. Poster at 9th World Congress on pain in Vienna. August 1999.

N Clayton, J Francis, R Munglani, S Collins, C Bountra The Novel Sodium Channel Blocker 4030W92 reduces the fall in CGRP expression in the dorsal horn of the spinal cord and prevents the development of mechanical allodynia following chronic constriction injury (CCI) in the rat. Poster at Pain Society meeting Edinburgh April 1999

N. Cougnon-Aptel, R Munglani, N.M. Clayton, P. Ward, and C Bountra. (1998) Changes in spinal cord neuropeptides in the adjuvant model of chronic inflammatory hyperalgesia, in the presence and absence of the neurokinin 1 (NK1) receptor antagonist GR205171 Br J Pharmacol 126, 285P

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Collins S.D., Clayton N.M, Nobbs M and Bountra C. (1997) The effect of 4030W92, a novel sodium channel blocker on the treatment of neuropathic pain in the rat. Br J Pharmacol, 123, 16p

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Clayton N M, Oakley I, Thompson S, Wheeldon A, Sargent B and Bountra C. (1997). Validation of the dual channel weight averager as an instrument for the measurement of clinically relevant pain. Br J Pharmacol, 120 P78

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Clayton NM, Trevethick MA, Strong P, Harman L (1991). Inhibition of indomethacin-induced gastric antral ulceration by the selective EP_3 receptor prostanoid GR63799 in the re-fed rat. 7th International Conference on Experimental Ulcer. Digestion Abstracts, P60.

Clayton NM, Trevethick MA and Strong P. (1991). Eicosanoids release from the antral and corpus regions of the rat stomach. Br J Pharmac, 102, 362P.

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Exhibit B

Figure 1

A combination of EP4 ligand and COX-2 ligand produced synergy

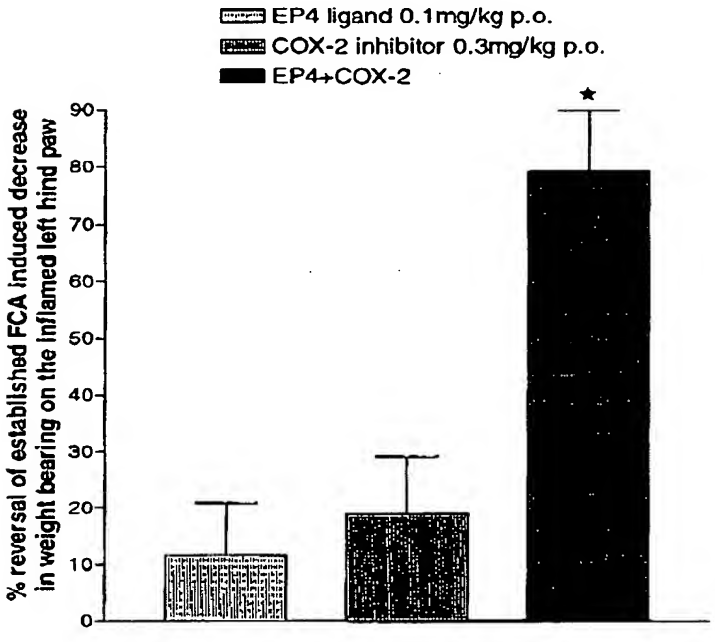

Figure 2

The combination of EP4 ligand and COX-2 inhibitor produced a synergistic effect